Showa Univ J Med Sci **22**(2), 85～93, June 2010

Original

Parallel Activation of the Amygdala and Visual Cortex Estimated by Dipole Tracing Analysis during Visual Stimulation of Fear and Sadness

Hironori SATOH, Yuri MASAOKA, Hideyo KASAI,
Takeshi KURODA, Lena AKAI, Mitsuru KAWAMURA
and Ikuo HOMMA

Abstract: In this study we used the electroencephalograph (EEG) dipole tracing method to analyze the visual and emotional evoked potentials (VEEPs) triggered by emotional stimuli induced by pictures of fear, sadness and happiness selected from the International Affective Picture System. We hypothesized that if we used the emotional pictures as triggers for averaging the EEG, we could determine VEEPs, and dipoles could be estimated in the visual cortex as well as in the areas related to the picture-induced emotions. We found the VEEP components elicited by fearful and sad stimuli were quite similar and there were no differences in the root mean square values of the positive waves, P1 and P2, in these two stimuli. However, the VEEP elicited by the happy stimulus had a significantly different amplitude compared to the fearful and sad stimuli. Different amplitude components of VEEPs between negative and positive emotions might be caused by differences in the processing of activations. The negative emotions of fear and sadness activated the amygdala in parallel with the visual cortex immediately after the stimuli; and at a later time period the anterior cingulate cortex was activated for conscious awareness of the negative emotions. A simple happy stimulus does not need parallel activation of the amygdala and anterior cingulate cortex, along with activation of the visual cortex. We suggest that parallel processing in the visual cortex and amygdala might serve to rapidly evaluate stimuli, in readiness for the conscious awareness of negative emotions.

Key words: amygdala, fear, sadness, visual cortex, visual stimuli

Introduction

Neuroimaging studies have shown the anatomical brain regions related to various emotions induced by visual stimuli[1-3]. These studies using functional magnetic resonance imaging and positron emission tomography have good space resolution and identify the areas of

Department of Physiology II, Showa University School of Medicine, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan.

the brain for cognition and emotions; however, these methods cannot accurately detect the time-to-time process of cognition and emotion brought on by visual stimuli. The electroencephalograph (EEG) dipole tracing (DT) method has been developed to localize dipoles estimated from event-related potentials using a brain model and to visualize the point-like dipoles in anatomical brain regions[4,5]. An advantage of dipole fitting is that it enables us to locate the dipole in millisecond intervals; therefore, we are able to see dipole movement to detect the process of brain activation. Dipole analysis has been used to locate the generators of visually evoked potentials (VEP) to pattern onset stimuli[6] and dipoles in the occipital lobe. The DT method not only estimates the areas related to simple visual stimuli but also estimates the brain regions associated with emotions stimulated by visual stimuli. Yoshimura *et al*[7] used facial expressions showing fear, disgust and happiness as visual stimuli and investigated the areas related to these emotions, estimated from VEP triggered by the onset of picture presentation. This study showed that the amygdala (AMG) is involved in the response to fearful expressions and that there is a difference in processing the fear response between normal subjects and patients with Parkinson's disease.

The International Affective Picture System (IAPS) has been used for visual stimuli to investigate the effect of visual emotional stimuli on physiological responses[8,9]. In this study, we analyzed the DT of VEPs triggered by emotional stimuli induced by pictures of fear, sadness and happiness selected from the IAPS. We hypothesized that if we used the emotional pictures as triggers for averaging the EEG, we could determine the visual and emotional evoked potentials (VEEPs), and dipoles could be estimated in the visual cortex as well as in the areas related to the picture-induced emotions. In addition, the time resolution of DT enabled us to detect any differences between the processing of negative emotions such as fear and sadness and the processing of happiness.

Methods

Subjects

Seven healthy subjects (mean age, 37.2 years) participated in this study; all subjects signed an informed consent form. This study was approved by the Ethics Committee of the Showa University School of Medicine.

Experimental paradigm

A three-stimulus oddball paradigm was used. Affective pictures (fear, sadness and happiness) were selected from the IAPS according to Lang *et al*[10] Using three pictures for fear, sadness and happiness, subjects were selected with high emotional scores measured with the Visual Analogue Scale (VAS; 20 cm of a horizontal line with the far left representing "feel nothing" and the far right "most unpleasant, sad or happy"). VAS averages for fear, sadness and happiness are indicated in Fig. 1. As in our previous study on the role of the amygdala in fear[7], we defined the fearful pictures as the target stimuli.

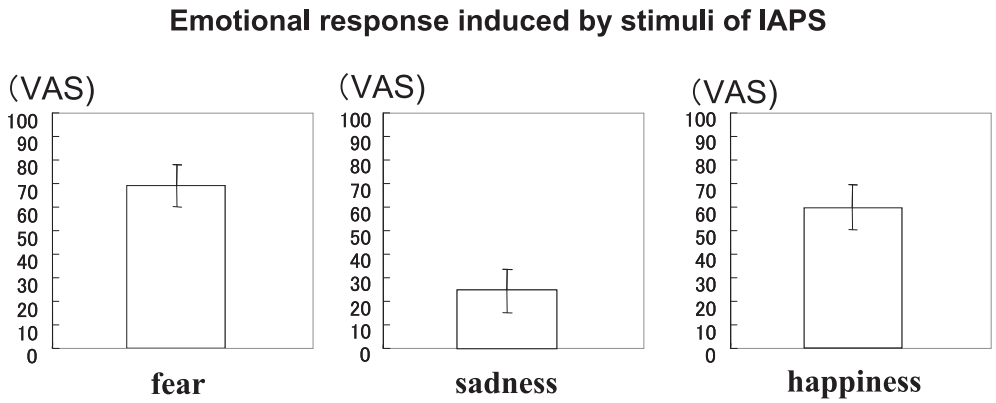

Fig. 1. Emotional levels were confirmed by the Visual Analogue Scale (VAS). The data indicate the means and standard deviations of all the subjects. The scale consisted of a 20 cm horizontal line with the left end indicating "feel nothing" and the right and indicating "most fearful, sad or happy".

Fearful, sad and happy pictures were presented for 500 ms, with a probability of 0.1, 0.1, and 0.8, respectively, in randomized order at intervals of 1500 ms. The order of stimulus presentation was controlled by a personal computer (1829-63J, IBM, USA) and stimuli were delivered through a head-mounted display (FX601, DAEYANG E&C, Korea). Subjects were instructed to keep their eyes open and to fix their gaze on the head-mounted display. Subjects were asked to press a button with his or her right index finger as soon as a target stimulus appeared on the screen.

Data acquisition

Nineteen electrodes were arranged according to the 10-20 system with the reference electrode attached to the right earlobe. Electro-oculograms (EOG) were recorded with electrodes placed at the inferior lateral cantus and supraorbitally to the left eye. Electrode impedances were held below 5 KΩ throughout the EEG recording. Potentials were measured with an EEG recorder (EEG-1100, Nihon Kohden, Japan) at 1000 Hz intervals and stored on magnetic optical disks for off-line analysis. Potentials were digitally filtered by a band pass-filter (5-100 Hz).

For off-line analysis of data, potentials, triggered by the onset of each emotional picture, were averaged so there were three averaged potentials corresponding to the three stimuli (fear, sadness and happiness) for each subject. Potentials from 100 ms pre- and 500 ms post-stimulus were averaged. We excluded noise from blinking and body movements (>50 μV).

Dipole tracing method

The averaged EEG potentials were transferred to dipole tracing software (Brain Space

Navigator ; BS-navi, Brain Research and Development, Japan) to estimate the location of the source generator. Details of the dipole tracing method using a scalp-skull-brain head model from the Montreal Neurological Institute (MNI) have been reported elsewhere[11, 12]. Locations of dipoles were estimated from the grand-averaged potentials across all the subjects incorporated with the standard head model created from a MNI brain template, which serves as the most common stereotaxic platform. We confirmed that the dipole locations estimated from the grand-averaged potentials with the MNI brain head model corresponded to specific brain areas which have already been documented[11, 12]. Goodness of fit of 98% or higher is considered to be significant for current-source generators.

Data analyses

VEEP, as described in the results, were composed of two positive waves ; the positive wave ranging from 150 ms to 200 ms is referred to as P1, and the second positive wave ranging from 250 ms to 300 ms is referred to as P2. Differences in root mean square (RMS) values of P1 / P2 between the three emotional stimuli were analyzed using a one-way analysis of variance (ANOVA) and post hoc multiple comparisons were performed using the Bonferroni test. For DT results, the activated areas for each subject were expressed as dipolarity of more than 98% and were computed automatically in each brain region. The total number of subjects who converged in the various brain regions was calculated. Specific anatomical regions for pre P1 were analyzed using a two-way ANOVA [factors : stimulus (fear, sadness, happiness) and anatomical region]. Similarly, separate two-way ANOVAs were conducted for specific anatomical regions for P1 and P2.

Results

VEEPs recorded from the Cz electrode, triggered by stimuli of fear (black line), sadness (gray line) and happiness (dark gray line), are shown on the left of Fig. 2. All Cz potentials are superimposed. Two Cz components, the amplitudes of P1 and P2 of the happy stimulus, were larger than those of the fearful and sad stimuli. RMS values of PI and P2 were also significantly higher for the happy stimulus than for the other two stimuli (Fig. 2, right panel, Fig. 3, $P < 0.05$).

The number of subjects with dipoles located in each anatomical region is shown in Table 1. Anatomical areas of interest have been described in our previous study[7]. Subjects with goodness of fit of 98% or higher were considered significant. Because the DT method detects moving dipoles over time after a triggering point, we analyzed the dipole locations for each of the three components (pre P1, P1 and P2). Analysis of the pre P1 component revealed there was a significant effect of the different stimuli ($P < 0.05$) as well as the anatomical region ($P < 0.01$). Post hoc multiple comparison testing showed a significant dipole location in the right AMG for the fear and sadness stimuli. For P1, there was no significant effect of the different stimuli ($P > 0.1$), but there was a significant effect of the

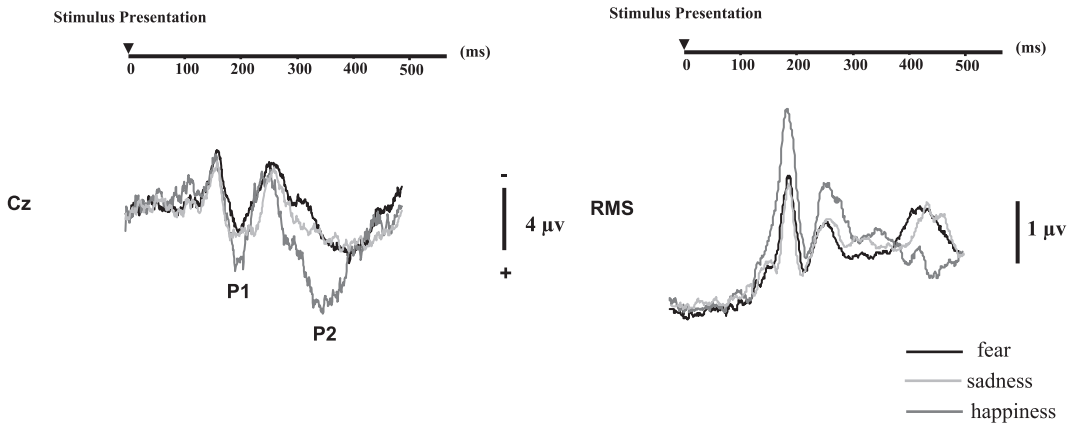

Fig. 2.
Left panel: Visual and emotional evoked potentials (VEEPs), recorded from the Cz electrode of the electroencephalograph, triggered by stimuli of fear (black line), sadness (gray line) and happiness (dark gray line). All Cz potentials are superimposed.
Right panel: Root mean square (RMS) values of averaged potentials triggered by each stimulus: fear (solid line), sadness (gray line) and happiness (dark gray line).

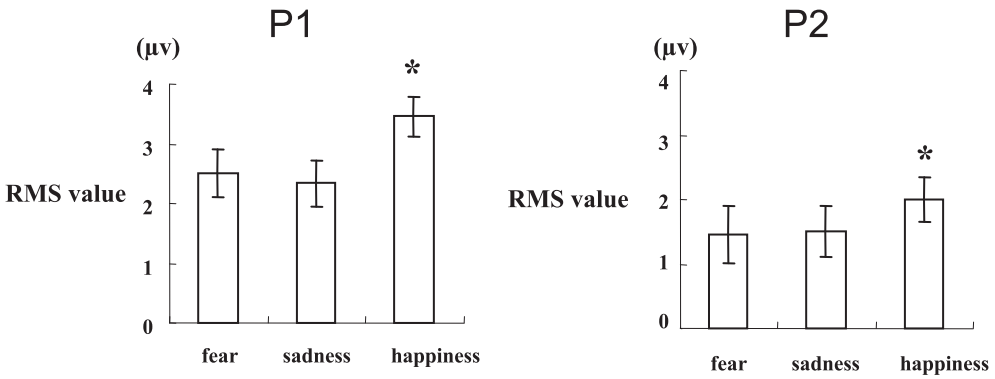

Fig. 3. Comparison of root mean square (RMS) values for P1 / P2 between the three stimuli. RMS values of P1 and P2 are significantly higher for the happy stimulus ($P < 0.05$).

anatomical region ($P < 0.01$). Post hoc multiple comparison testing showed significant dipole locations in the specific anatomical regions of the left and right AMG for the fearful and sad stimuli and occipital gyrus (OcG) for all stimuli ($P < 0.01$). For the P2 component, there was a significant effect of stimuli ($P < 0.01$) and anatomical region ($P < 0.01$). Post hoc multiple comparison testing showed that the OcG bilaterally were significantly activated for the happy stimulus, while for the fearful and sad stimuli dipoles were significantly converged in the bilateral anterior cingulate cortex.

Dipole locations estimated from the grand-averaged potentials for each of the three stimuli were superimposed on the MNI brain model, and shown in Fig. 4.

Table 1. Dipole locations for pre P1, P1 and P2

Pre P1 (range from 0 ms to 150 ms)

	Temporal lobe												Parietal lobe						Others					
	Fusi		ITG		MTG		STG		Para HC		AMG		OcG		AG		Precune		A cingulate		Retro		Cbll	
	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R
fear	0	0	1	2	2	3	1	1	3	5	3	7*	0	0	1	1	0	1	0	0	1	0	0	0
sadness	0	0	1	1	2	1	1	1	2	4	3	8*	0	0	0	0	1	1	0	0	0	0	0	0
happy	0	0	1	1	2	3	2	3	4	2	0	0	0	0	0	0	1	1	0	0	1	1	0	0

P1 (range from 150 ms to 300 ms)

	Temporal lobe												Parietal lobe						Others					
	Fusi		ITG		MTG		STG		Para HC		AMG		OcG		AG		Precune		A cingulate		Retro		Cbll	
	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R
fear	0	1	0	1	1	4	2	2	5	3	7*	7*	8*	8*	7	6	5	5	1	3	0	0	0	0
sadness	0	2	1	1	2	4	1	3	2	3	8*	8*	8*	8*	6	6	7	7	2	3	0	0	0	0
happy	0	0	1	2	0	3	1	1	1	1	3	3	8*	8*	6	6	7	7	2	1	0	0	0	0

P2 (range from 300 ms to 350 ms)

	Temporal lobe												Parietal lobe						Others					
	Fusi		ITG		MTG		STG		Para HC		AMG		OcG		AG		Precune		A cingulate		Retro		Cbll	
	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R
fear	0	0	0	0	1	1	2	2	0	0	0	3	3	2	1	2	1	3	7†	8†	2	2	2	1
sadness	0	0	0	0	2	1	1	1	0	0	0	4	2	1	1	1	1	3	6†	7†	2	3	1	1
happy	0	0	0	0	1	1	1	0	0	0	0	0	7†	6†	3	1	1	2	0	0	1	1	2	1

L = left, R = right

AMG, amygdala; AG, angular gyrus; Cbll, cerebellum; Fusi, fusiform gyrus; Para HC, parahippocampal gyrus; ITG, inferior temporal

Gyrus; MTG, middle temporal gyrus; precune, precuneus; retro, retrosplenial cortex; OcG, occipital gurus; STG, superior temporal gyrus

*Significant anatomical regions estimated by one dipole analysis.

†Significant anatomical regions estimated by two dipole analyses.

Discussion

We measured VEEPs during affective pictures of fear, sadness and happiness selected from the IAPS and investigated differences in the generators of each VEEP, during sequential time periods after the stimulus onset. We discuss the differences in cognitive and emotional processing between negative emotions (fear and sadness) and positive emotions, as indicated by our results which focus on the differences in the P1 and P2 components and dipole locations.

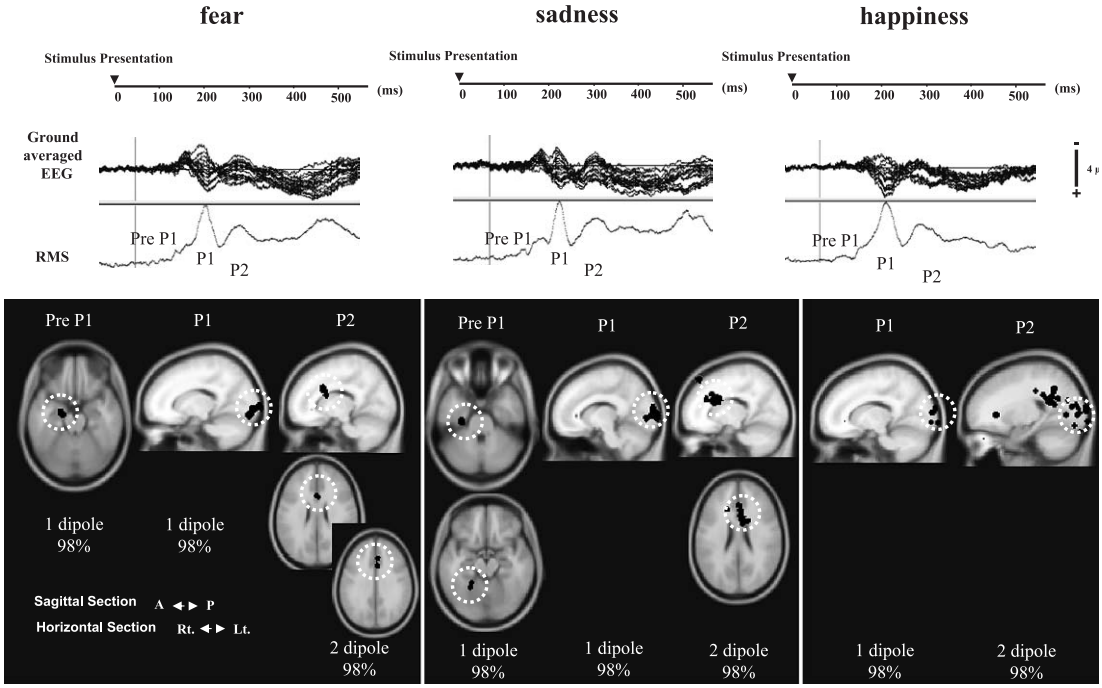

Fig. 4. Dipole locations estimated from the grand-averaged potential for each of the three stimuli were superimposed on the Montreal Neurological Institute (MNI) brain model.

Differences in VEEP features between fearful / sad and happy stimuli

The VEEP components which were elicited by fearful and sad stimuli were quite similar, and we found there were no differences in the RMS values of P1 and P2 for these two stimuli. However, the VEEP elicited by the happy stimulus had a significantly different amplitude compared to the fearful and sad stimuli. This difference might be due to different processing of brain activations, as reflected in our DT analysis.

AMG activation parallels activation in the OcG

For the P1 component, the common brain area highlighted by all three stimuli was the OcG, which is not surprising since the visual signal primarily reaches the OcG in the time range of 150 ms to 300 ms[6, 7]. However, it is interesting to find that the right AMG was activated before activation of the OcG for the fearful and sad stimuli. Functional neuroimaging studies have investigated the neuroanatomical correlates for the negative emotions of fear and anxiety[13, 14]. These studies have revealed that the AMG plays a crucial role in processing these negative emotions. Our new finding is that the AMG is activated in parallel with processing in the OcG. Morris and colleagues have shown the right AMG responds to unseen emotional stimuli and suggested that there is a subcortical pathway to the right AMG, via the midbrain and thalamus[15]. It is suggested that this route enables

processing of unseen visual events in parallel with a cortical route which is necessary for conscious identification. In our study, in the pre P1 stage, the subjects were not able to be consciously aware of the stimuli, but parallel processing in the OcG might serve to rapidly evaluate stimuli in readiness for the conscious awareness of negative emotions. Interestingly, Morris *et al* noted that the subcortical pathway implicated in the processing of unseen stimuli parallels animal models of fear conditioning[15]. Unconscious evaluation of stimuli might be needed in readiness for a precise emotional response.

At P2, which ranged from 250 ms to 300 ms, the anterior cingulate gyrus was activated with the fearful and sad stimuli while only the OcG remained activated with a happy stimulus. There are several reports showing anterior cingulate gyrus responses to negative emotions such as fear, anxiety, sadness and pain[16, 17], thus it is reasonable to conclude that this area responded to fearful and sad emotions in our study. Only the OcG was activated with the happy stimulus, therefore activation of the AMG and anterior cingulate gyrus might not be necessary with this stimulus. Different VEEP amplitude components between negative and positive emotions might be caused by differences in the processing of these activations; that is, a simple happy stimulus may not need parallel activation of the AMG and anterior cingulate gyrus along with activation of the OcG. A happy stimulus does not need these extra areas of activation and so simple visual stimulation activates the OcG more strongly. This is reflected in the large amplitude and RMS value for the happy stimulus.

In summary, pictures selected from the IAPS affect emotional changes. These changes were correlated with brain activations. In particular, the negative emotions of fear and sadness activated the AMG in parallel with the OcG immediately after the stimuli, and at a later time period the anterior cingulate cortex was activated for conscious awareness of these negative emotions. These results were obtained in normal subjects, however this research may apply to patients who have impaired social interactions, and may help to improve our understanding of their level of cognitive and emotional response.

References

1) Lane RD, Reiman EM, Ahern GL, Schwartz GE and Davidson RJ: Neuroanatomical correlates of happiness, sadness, and disgust. *Am J Psychiatry* **154**: 926–933 (1997)
2) Phan KL, Wager T, Taylor SF and Liberzon I: Functional neuroanatomy of emotion: a meta-analysis of emotion activation studies in PET and fMRI. *Neuroimage* **16**: 331–348 (2002)
3) Itier RJ and Batty M: Neural bases of eye and gaze processing: the core of social cognition. *Neurosci Biobehav Rev* **33**: 843–863 (2009)
4) Homma I, Masaoka Y, Hirasawa K, Yamane F, Hori T and Okamoto Y: Comparison of source localization of interictal epileptic spike potentials in patients estimated by the dipole tracing method with the focus directly recorded by the depth electrodes. *Neurosci Lett* **304**: 1–4 (2001)
5) Masaoka Y, Koiwa N and Homma I: Inspiratory phase-locked alpha oscillation in human olfaction: source generators estimated by a dipole tracing method. *J Physiol* **566**: 979–997 (2005)
6) Ikeda H, Nishijo H, Miyamoto K, Tamura R, Endo S and Ono T: Generators of visual evoked potentials investigated by dipole tracing in the human occipital cortex. *Neuroscience* **84**: 723–739 (1998)

7) Yoshimura N, Kawamura M, Masaoka Y and Homma I : The amygdala of patients with Parkinson's disease is silent in response to fearful facial expressions. *Neuroscience* **131** : 523-534 (2005)
8) Bradley MM, Cuthbert BN and Lang PJ : Pictures as prepulse : attention and emotion in startle modification. *Psychophysiology* **30** : 541-545 (1993)
9) Amrhein C, Mühlberger A, Pauli P and Wiedemann G : Modulation of event-related brain potentials during affective picture processing : a complement to startle reflex and skin conductance response? *Int J Psychophysiol* **54** : 231-240 (2004)
10) Lang PJ, Bradley MM and Cuthbert BN : *International Affective Picture System (IAPS): instruction manual and affective ratings. Technical Report A-4.* Center for Research in Psychophysiology, University of Florida, Gainesville, FL (1999)
11) Inoue M, Masaoka Y, Kawamura M, Okamoto Y and Homma I : Differences in areas of human frontal medial wall activated by left and right motor execution : dipole-tracing analysis of grand-averaged potentials incorporated with MNI three-layer head model. *Neurosci Lett* **437** : 82-87 (2008)
12) Masaoka Y, Yajima H, Takayama M, Kawase A, Takakura N and Homma I : Typical dipole locations can be estimated using averaged somatosensory-evoked potentials and a standard brain model. *J Physiol Sci* **59** : 299-305 (2009)
13) Adolph R, Tranel D, Damasio H and Damasio AR : Impaired recognition of emotion in facial expressions following bilateral damage to the human amygdala. *Nature* **372** : 669-672 (1994)
14) Morris J, Friston KJ, Büchel C, Frith CD, Young AW, Calder J and Dolan RJ : A neuromodulatory role for the human amygdala in processing emotional facial expressions. *Brain* **121** : 47-57 (1998)
15) Morris J, Öhman A and Dolan RJ : A subcortical pathway to the right amygdala mediating "unseen" fear. *Proc Natl Acad Sci USA* **96** : 1680-1685 (1999)
16) Devinsky O, Morrell MJ and Vogt BA : Contributions of anterior cingulate cortex to behaviour. *Brain* **118** : 279-306 (1995)
17) Vogt B : Pain and emotion interactions in subregions of the cingulate gyrus. *Nat Rev Neurosci* **6** : 533-544 (2005)

[Received January 19, 2010 : Accepted February 4, 2010]